Vet school deja vu: practical reproductive physiology with emphasis on estrus and synchronization of ovulation

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Estrus biology has implications for reproductive management

- Effect of early pregnancy loss on expression and detection of estrus

- Expression of estrus during early lactation (VWP) is an indicator of early lactation outcomes and a predictor of reproductive performance
  - Might be used as indicator of good management and predictor for targeted management
Biological events that occur during pregnancy affect return to estrus
Maternal Recognition of Pregnancy

Hormone Concentration

PGF$_{2\alpha}$

Conceptus

Ovulation

Days

Estrus

5

10

15

Estrus

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Maternal Recognition of Pregnancy

- Estrus
- Hormone Concentration
- Days
- Conceptus
- Ovulation
- Interferon-τ

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Pregnancy Loss Affects Ovarian Function

Evidence of Embryo presence based on ISG and PSPB 62% (37/63)

Days after AI

Wijma et al., 2016 (BOR 95(5):112)
Pregnancy Loss Affects Ovarian Function

Evidence of Embryo presence based on ISG and PSPB
62% (37/63)

Evidence of Embryo presence based on ISG and PSPB
37% (23/63)

Preg Loss 38% (14/37)

Days after AI

Wijma et al., 2016 (BOR 95(5):112)
Cows with Early Embryo Mortality (<22 d after AI) return to estrus earlier

Wijma et al., 2016 (BOR 95(5):112)
Cows with Late Embryo Mortality (>22 d after AI) return to estrus later

Wijma et al., 2016 (BOR 95(5):112)
Previous AI

No ovulation after previous AI in spite of normal follicular waves. CL present from previous AI (ovu)

No ovulation before NPD and no CL present form previous AI (anovular o proestrus)

NPD 32 ± 3 d

Ovulation before NPD
Estrus biology has implications for reproductive management

- Effect of early pregnancy loss on expression and detection of estrus
- Expression of estrus during early lactation (VWP) is an indicator of early lactation outcomes and a predictor of reproductive performance
  - Might be used as indicator of good management and predictor for targeted management
Monitored estrus during the VWP with automated estrus alert (AEA) system

AEA = automated estrus alert

21 DIM  49 DIM

**ESTRUS** before 50 DIM (VWP)

54.2%  (705/1,300)

**NO ESTRUS** before 50 DIM (VWP)

45.7%  (595/1,300)

Rial et al., 2021 J. Dairy Sci. Volume 104, E-Supplement 1
Cows with AEA during VWP more likely to be AIE, conceive at 1st AI, and be preg. at 150 DIM

<table>
<thead>
<tr>
<th>GROUP</th>
<th>E-VWP</th>
<th>NOE-VWP</th>
<th>% points diff.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows AIE, %</td>
<td>84.9</td>
<td>48.5</td>
<td>36.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>(416/490)</td>
<td>(197/406)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All P/AI, %</td>
<td>47.7</td>
<td>42.2</td>
<td>5.5</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(331/693)</td>
<td>(247/585)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preg 150 DIM</td>
<td>84.9</td>
<td>66.7</td>
<td>18.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>(390/459)</td>
<td>(320/417)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AEA = automated estrus alert

Rial et al., 2021 J. Dairy Sci. Volume 104, E-Supplement 1
Monitored cows based on automated estrus alert (AEA) during the VWP

AEA = automated estrus alert

15 DIM

49 DIM

ESTRUS before 50 DIM (VWP)
59.7% (507/849)

NO ESTRUS before 50 DIM (VWP)
40.3% (342/849)
Cows with AEA during VWP more likely to be AIE and had greater P/AI to 1st service

<table>
<thead>
<tr>
<th>GROUP</th>
<th>E-VWP (%)</th>
<th>NOE-VWP (%)</th>
<th>% points diff.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows AIE, %</td>
<td>87.9 (218/248)</td>
<td>42.5 (68/160)</td>
<td>45.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>All P/AI, %</td>
<td>43.1 (107/248)</td>
<td>23.2 (37/160)</td>
<td>19.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Preg 150 DIM</td>
<td>73.8 (107/145)</td>
<td>57.3 (55/96)</td>
<td>16.5</td>
<td>0.02</td>
</tr>
</tbody>
</table>

AEA = automated estrus alert
Proportion of cows with automated estrus alerts (AEA) during the VWP

AEA = automated estrus alert

 Estrus before 50 DIM (VWP)  
56.4% (507/2,149)

 No Estrus before 50 DIM (VWP)  
43.6% (937/2,149)
Cows with fewer risk factors were more likely to express estrus and express estrus earlier during the VWP than cows with more risk factors.

- 0RF = 25.0% (296/1,184)
- 1RF = 40.2% (476/1,184)
- 2+RF = 34.8% (412/1,184)
Ovarian Physiology During Ovsynch

7 days

GnRH

56 h

PGF$_{2\alpha}$

16 h

GnRH

TAI

Pursley et al. 1995
Ovarian Physiology During Ovsynch

Pursley et al. 1995
Critical steps and associated issues in synchronization protocols

High P4

Lack of CL ~5-35%

Ovulatory failure ~25-60%

Low P4 & High E2

No CL Regre. ~10-25%

Ovulatory failure ~5-10%

GnRH

PGF$_{2\alpha}$

GnRH

TAI

7d

56h

16h

Ovulatory failure ~25-60%

Lack of CL ~5-35%
Strategies for optimizing TAI programs

1. High P4 during fol. growth – endogenous vs. exogenous P4

   - Lack of CL ~5-35%
   - Ovulatory failure ~25-60%

   - High P4
   - Low P4 & High E2

   - No CL Regre. ~10-25%

   - GnRH
   - PGF$_{2\alpha}$
   - GnRH
   - TAI

2. Increase response to GnRH 1

3. Increase CL regression rate & minimize P4 levels
Fertility programs include presynchronization of the estrous cycle to optimize P/Al

1. High P4 during fol. growth – endogenous vs. exogenous P4

Presynch-Ovsynch (Moreira et al., 2001)
- 14 days
  - PGF$_{2\alpha}$
- 12 days
  - GnRH

Double-Ovsynch (Souza et al., 2008)
- 7 days
  - PGF$_{2\alpha}$
- 3 days
  - GnRH
- 7 days
  - PGF$_{2\alpha}$

G6G (Bello et al., 2006)
- 2 days
  - PGF$_{2\alpha}$
- 6 days
  - GnRH

First Service TAI
- 7 days
- 56 h
- 16 h
- PGF$_{2\alpha}$
- GnRH
- TAI

Progestrone (ng/mL) at G1
- 21% of cows
  - 38%, 41%, 40%, 42%, 38%, 37%
- 6% of cows
  - 30%

Carvalho et al., 2015
Physiology Double-Ovsynch

GnRH  PGF$_{2\alpha}$  GnRH  GnRH  GnRH  PGF$_{2\alpha}$  GnRH  TAI
Pre-Ovsynch  7 d  3 d  7 d  7 d  56h  16h  Breeding-Ovsynch

Souza et al., 2008; Giordano et al., 2012; Herlihy et al., 2012; Giordano et al., 2013
Fertility programs include presynchronization of the estrous cycle to optimize P/AI

1. High P4 during fol. growth – endogenous vs. exogenous P4

Presynch-Ovsynch (Moreira et al., 2001)
- 14 days
- 12 days

Double-Ovsynch (Souza et al., 2008)
- 7 days
- 3 days
- 7 days

G6G (Bello et al., 2006)
- 2 days
- 6 days

High P4

First Service TAI

GnRH

PGF$_{2\alpha}$

GnRH

7d

56h

16h

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Fertility programs include presynchronization of the estrous cycle to optimize P/AI

High P4 during fol. growth – endogenous vs. exogenous P4

Presynch-Ovsynch (Moreira et al., 2001)
- 14 days
- 12 days

Double-Ovsynch (Souza et al., 2008)
- 7 days
- 3 days
- 7 days

G66 (Bello et al., 2006)
- 2 days
- 6 days

GnRH

PGF$_{2\alpha}$

GnRH

P4 & LH pulses

First Service TAI

High progesterone

Low progesterone

GnRH

PGF$_{2\alpha}$

GnRH

TAI

7d

56h

16h
Fertility programs include presynchronization of the estrous cycle to optimize P/AI

1. High P4 during fol. growth – endogenous vs. exogenous P4
Fertility programs include presynchronization of the estrous cycle to optimize P/AI

1. High P4 during fol. growth – endogenous vs. exogenous P4
P4 levels affect ovulatory response to GnRH

Giordano et al., 2013

P4 levels affect ovulatory response to GnRH

Ovulatory response $P < 0.05$

>90% cows fol $\geq$ 10 mm

Giordano et al., 2013

Progesterone concentration at G1:
- Low: 60% (134/227)
- Medium: 40% (115/302)
- High: 10% (33/211)

Ovulatory Response to G1 (%)

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Effect of P4 on LH response to GnRH

100 μg of GnRH

- High P4 Env
- Low P4 Env

P4 Env $P < 0.001$
Time $P < 0.001$
P4 Env X Time $P < 0.001$

Giordano et al., 2013
Model of P4 effect on LH release

High P4 Environment

(-)

Ovulatory response

Low P4 Environment

Ovulatory response

Presynchronization 7 days 56 h 16 h

GnRH PGF$_{2\alpha}$ GnRH TAI
Strategies for optimizing ovulatory response to 1st GnRH

2. Increase response to GnRH 1

- Greater GnRH dose
- Induce partial CL regression
- hCG?

Presynch
Ovsynch
PG3-G
G-6-G
hCG
GnRH

GnRH
PGF$_{2\alpha}$
GnRH
TAI

Presynchronization 7d 56h 16h

65-95% with CL
90-95% Fol ≥ 10 mm

Fol ≥ 10 mm
Greater doses of GnRH increased GnRH-induced LH surge

![Graph showing LH levels over time after GnRH injection for different doses of GnRH.](image)

High Progesterone

- 100 μg GnRH
- 200 μg GnRH

Dose $P < 0.001$
Time $P < 0.001$
Dose X Time $P < 0.001$

Giordano et al., 2013
Greater GnRH dose increased ovulation in a P4 concentration dependent manner.

Greater dose of GnRH did not result in greater P/AI ($P = 0.24$)

- 100 ug = 42.2% (n = 533)
- 200 ug = 44.1% (n = 551)
Explored effect of GnRH dose on fertility to TAI

CRD to evaluate effect of 100 vs 200 ug of **Gonadorelin hydrochloride**

Lactating Holstein cows – 1\textsuperscript{st} TAI at 64-75 primi (n = 720), 59-70 multi (n = 1,192)

Conventional semen only

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Valdes-Arciniega et al., 2020 (JDS Abstract)
Effect of GnRH (Gonadorelin hydrochloride) dose on P/AI - Primiparous cows

Valdes-Arciniega et al., 2020 (JDS Abstract)
Effect of GnRH (Gonadorelin hydrochloride) dose on P/Al – Multiparous cows

Valdes-Arciniega et al., 2020 (JDS Abstract)
Optimizing luteolysis before TAI increases fertility

3 Increase CL regression rate & minimize P4 levels

GnRH ➔ PGF$_{2\alpha}$ ➔ GnRH ➔ TAI

| 7d | 56h | 16h |

PGF$_{2\alpha}$ levels:
- $\geq 2$ ng/ml
- $< 0.5$ ng/ml

Progesterone (ng/mL) at G2:
- 14% of cows

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Type of CL present at induction of luteolysis affects the response to PGF

- Greater PGF dose
- Sequential PGF treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL regression (&lt;1 ng/mL)</td>
<td>Old CL 91\textsuperscript{a} (47)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Old+New CL 97\textsuperscript{a} (48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New CL only 81\textsuperscript{b} (73)</td>
<td></td>
</tr>
</tbody>
</table>

Type of CL presents affects response to PGF

GnRH

PGF\(_{2\alpha}\)

GnRH

TAI

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Sequential PGF treatments (two/24 h) increased CL regression risk

**Table 1. Effect of 1 versus 2 treatments with prostaglandin F$_{2\alpha}$ (PGF) on percentage of cows with complete regression of the corpus luteum in primiparous and multiparous cows synchronized with Double-Ovsynch (experiment 1)**

<table>
<thead>
<tr>
<th></th>
<th>1 PGF (%)</th>
<th>2 PGF (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>81.2 (65/80)</td>
<td>97.5 (77/79)</td>
<td>0.001</td>
</tr>
<tr>
<td>Multiparous</td>
<td>84.4 (81/96)</td>
<td>96.7 (86/89)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.69</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>83.0 (146/176)</td>
<td>97.0 (163/168)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

1. Circulating progesterone <0.5 ng/mL at 56 h after first PGF in cows that had ≥2.0 ng/mL of progesterone on the day of first PGF.

2X vs 1X PGF increased CL reg. ~14p.p.
Sequential PGF treatments (two/24 h) increased P/AI

Table 6. Effect of treatment with a second prostaglandin F subalpha (PGF sub2alpha) (PGF) on P/AI during the Ovsynch (experiment 2) or Double-Ovsynch (experiment 1) protocols.

<table>
<thead>
<tr>
<th>Parity</th>
<th>1 PGF, % (no./no.)</th>
<th>2 PGF, % (no./no.)</th>
<th>Effect of PGF difference, % (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>39.3 (140/356)</td>
<td>40.6 (139/342)</td>
<td>+3.31% (0.39)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>32.5 (296/910)</td>
<td>36.5 (333/913)</td>
<td>+12.31% (0.043)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.04</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>34.4 (436/1256)</td>
<td>37.6 (471/1251)</td>
<td>+9.45% (0.049)</td>
</tr>
</tbody>
</table>

1Results from experiments 1 and 2 were combined for the analysis with all cows assigned to the experiments included in the analysis.

Effect of 1X vs. 2X PGF

2X vs 1X PGF increased P/AI 4.0 p.p. multiparous only
Sequential PGF treatm. (two/24 h) increased CL regression rate

Meta-analysis: 2X vs 1X PGF increased CL reg. ~12 p.p.
Sequential PGF treatment (two/24 h) increased P/AI

<table>
<thead>
<tr>
<th>Manuscript</th>
<th>1PGF</th>
<th>2PGF</th>
<th>Relative risk</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barletta et al. (2018)</td>
<td>107/349</td>
<td>137/387</td>
<td>1.15</td>
<td>0.94-1.40</td>
<td>0.42</td>
</tr>
<tr>
<td>Brusveen et al. (2009)</td>
<td>78/197</td>
<td>88/182</td>
<td>1.22</td>
<td>0.70-1.45</td>
<td>0.96</td>
</tr>
<tr>
<td>Carvalho et al. (2015a)</td>
<td>150/462</td>
<td>168/435</td>
<td>1.19</td>
<td>0.93-1.45</td>
<td>0.32</td>
</tr>
<tr>
<td>Heidari et al. (2017)</td>
<td>49/149</td>
<td>54/144</td>
<td>1.14</td>
<td>0.90-1.39</td>
<td>0.37</td>
</tr>
<tr>
<td>Santos et al. (2016)</td>
<td>95/266</td>
<td>111/268</td>
<td>1.16</td>
<td>0.95-1.39</td>
<td>0.12</td>
</tr>
<tr>
<td>Wiltbank et al. (2015)</td>
<td>436/1,266</td>
<td>471/1,251</td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (fixed effects)</td>
<td>915/2,689</td>
<td>1,029/2,667</td>
<td>1.14</td>
<td>0.94-1.38</td>
<td>0.30</td>
</tr>
<tr>
<td>Total (random effects)</td>
<td>915/2,689</td>
<td>1,029/2,667</td>
<td>1.14</td>
<td>0.94-1.38</td>
<td>0.30</td>
</tr>
<tr>
<td>Pooled proportion (%)</td>
<td>34.0</td>
<td>38.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Meta-analysis: 2X vs 1X PGF increased P/AI ~4.6 p.p.

1. $I^2$ = proportion of total variation of effect size estimates that is due to heterogeneity.
2. Relative risk for conceiving at timed AI using a single PGF$_{2\alpha}$ treatment (1PGF) compared with 2 PGF$_{2\alpha}$ treatments (2PGF) during the Ovsynch protocol.
Endocrine status and stage of ovarian structure development at the time of key hormonal treatments affects the response to individual treatments and thus, whole protocol.

Equivocal evidence to support increasing dose of GnRH at beginning of breeding-Ovsynch to improve P/AI – effects might analog or salt dependent.

Supportive evidence for giving two doses of PGF 24 h apart and growing evidence that a double PGF dose before TAI might improve P/AI – effects might be parity specific.
This work was supported by the USDA National Institute of Food and Agriculture, Animal Health Program Project # 2017-67015-26772, Hatch project NYC-2020-21-255, and Multistate project 1021189. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the National Institute of Food and Agriculture (NIFA) or the United States Department of Agriculture (USDA).